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USSN: 09/844,501
Dkt. No.: 8325-0015
S15-US1
PATENT

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9/30/04 Michelle Hobson
Date Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Examiner: Jeffrey N. Fredman
WOLFFE et al.	Group Art Unit: 1634
Serial No.: 09/844,501	Confirmation No.: 9055
Filing Date: April 27, 2001	Customer No.: 20855
Title: DATABASES OF REGULATORY SEQUENCES; METHODS OF MAKING AND USING SAME	

TRANSMITTAL LETTER

Mail Stop Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313

Sir:

Transmitted herewith for filing, please find the following documents:

x Brief on Appeal with attached Appendix A through D (34 pages) *in triplicate*

x Return receipt postcard.

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	NO. OF CLAIMS	CLAIMS PREVIOUSLY PAID FOR	EXTRA CLAIMS	RATE	FEE
Total Claims	30	- 122	0	x $18.00	$0
Independent Claims	2	- 22	0	x $86.00	$0
Multiple dependent claims not previously presented, add $290.00					$0
Total Amendment Fee					$0
Fee for Filing an Appeal Brief					$330.00
Small Entity Reduction (if applicable)					$165.00
TOTAL FEE DUE					$165.00

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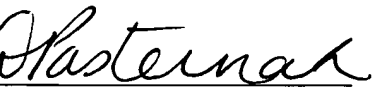
Respectfully submitted,

Date: September 30, 2004

By: 
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

A.P. Wolffe *et al.*

Application No.: 09/844,501

Filed: April 27, 2001

For: DATABASES OF REGULATORY SEQUENCES; METHODS OF MAKING AND USING SAME

Examiner: Jeffrey N. Fredman

Group Art Unit: 1637

Confirmation No.: 9055

BRIEF ON APPEAL

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

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Application No.: 09/844,501

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Examiner: Jeffrey N. Fredman

Group Art Unit: 1637

Confirmation No.: 9055

BRIEF ON APPEAL

Mail Stop Appeal Brief
Commissioner for Patents
Alexandria, VA 22313

Sir:

INTRODUCTION

Appellants submit in triplicate their brief on appeal in accordance with 37 C.F.R. §1.192. All claims were finally rejected under 35 U.S.C. § 103 in a Final Office Action mailed April 13, 2004. A Notice of Appeal was filed August 9, 2004, making a Brief on Appeal due on or before

October 9, 2004. Accordingly, this Brief is timely filed. Appellants respectfully request that the decision of the Examiner be reversed.

I. REAL PARTIES IN INTEREST

Sangamo BioSciences, Inc., the assignee of record of the above-referenced patent application is the real party in interest in this matter.

II. RELATED APPEALS AND INTERFERENCES

Appellants are not aware of any related appeals or interferences.

III. STATUS OF THE CLAIMS

Claims 123-152 are currently pending in the above-referenced case (hereinafter "the application"). The application was originally filed on April 27, 2001 with claims 1 to 122. Claims 1 to 122 were canceled and claims 123-152 were newly presented in a second preliminary amendment filed July 23, 2002 and were variously amended in papers filed December 17, 2003 and February 26, 2004. Following a telephone conference with the Examiner, Applicants amended the claims in a Supplemental Amendment filed June 16, 2004, to make explicit what was previously implicit. These amendments were not entered. Accordingly, claims 123-152 are pending as shown in Appendix A. All pending claims remain rejected under 35 U.S.C. § 103.

IV. STATUS OF THE AMENDMENTS

In response to the Examiner's Final Office Action mailed April 13, 2004, Appellants filed a Response on May 18, 2004. No amendments to the claims were made in that response.

Following receipt of an Advisory Action mailed June 2, 2004, a telephone call was placed to the Examiner to discuss potential claim amendments and the Examiner encouraged Appellant to present those amendments. As a result, Appellants filed a Supplemental Response After Final on June 16, 2004 in which independent claims 123 and 143 were amended to make explicit that the claimed libraries or collections comprise different sequences. A second Advisory Action was mailed on June 29, 2004, indicating that amendments to the claims would not be entered. Thus, all claims remained rejected for the reasons set forth in the Final Office Action.

V. SUMMARY OF THE CLAIMS

The claimed subject matter relates to methods for preparing libraries of regulatory DNA sequences and to methods of isolating collections of regulatory polynucleotide sequences. The methods are based on the fact that functioning transcriptional regulatory sequences are present, in the cell, in a chromatin structure that differs from the chromatin structure of non-regulatory DNA (such as, for example, coding sequences). One manifestation of this difference in chromatin structure is that regulatory sequences are more susceptible to cleavage by nucleases (*i.e.*, more accessible) than is bulk chromatin, which is packaged into nucleosomal structures. Thus, exposure of cellular chromatin to nucleases generally results in preferential destruction of regulatory sequences relative to bulk genomic DNA.

Although it has been possible to identify regulatory sequences by their hypersensitivity to, *e.g.*, nucleases, it has not heretofore been possible to isolate and purify such sequences, because they are destroyed in the process of being identified. The presently-claimed subject matter provides, for the first time, methods not only for identifying, but also for isolating and purifying these regulatory sequences. Moreover, the claimed methods allow the simultaneous purification of a plurality of regulatory sequences from a cell, making it possible to obtain DNA libraries which contain all of the regulatory DNA sequences that are active in a particular cell

type.[1] In particular, the methods of preparing a library typically comprise providing a cell nucleus comprising cellular chromatin (page 102, lines 15-18; page 112, line 33 through page 113, line 10); contacting the nucleus with a first enzyme that reacts with accessible regions of cellular chromatin (page 102, lines 30-33; page 113, line 11); deproteinizing the cellular chromatin to generate deproteinized DNA (page 49, lines 27-29; page 113, lines 14-16); contacting the deproteinized DNA with a second enzyme to generate DNA fragments (page 49, lines 27-29; page 113, lines 18-22); contacting the DNA fragments with a population of vector molecules, the vector molecules comprising a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends (page 50, lines 1-4; page 113, line 28 through page 114, line 5); and selecting polynucleotides comprising a DNA fragment ligated to a vector molecule (pages 50-51; page 114, lines 5-7). *See* also page 52, lines 3-15. Furthermore, during certain steps in the method, a cell nucleus can be embedded in agarose (page 49, lines 29-31; page 113, lines 12-24).

The cells from which the nucleus is obtained may be animal cells, plant cells and microbial cells. (page 56, line 2). In certain embodiments, a plurality of different libraries of regulatory DNA sequences are prepared, wherein each library is obtained from a different cell. The nuclei may be obtained from cells at different stages of development; cells in different tissues; diseased cells and counterpart normal cells; cells that express a gene of interest at different levels; and/or infected cells and counterpart uninfected cells (page 7, lines 31-34; page 47, lines 5-9).

In any of these methods, the first enzyme may a nuclease, such as DNAse I (page 48, line 7) or a restriction enzyme (page 113, line 11). In addition, a plurality of different libraries of regulatory DNA sequences may be prepared and, for each library, a different first enzyme is used (page 7, lines 29-31). The different libraries may be combined (page 47, lines 10-11). In certain embodiments, the DNAseI ends are converted to blunt ends (page 51, lines 3-6). Similarly, the

[1] This is in some ways analogous to microarray analyses, which identify all of the transcribed sequences (*i.e.*, expressed genes) in a cell. The presently-claimed methods identify all of the regulatory sequence (responsible for the transcription of those expressed genes) in the cell.

second enzyme may be a restriction enzyme, such as Sau3AI (page 49, lines 27-29; page 50, lines 4-8). In certain embodiments, the second end of the vector molecule is generated by digestion with BamHI (page 50, lines 1-5). The first end of the vector may be blunt-ended, for example as generated by digestion with EcoRV or SmaI (page 50, lines 1-4; page 50, line 7-8).

The claimed subject matter also relates to a method for isolating a collection of polynucleotides comprising cellular regulatory sequences, wherein the method comprises: contacting cellular chromatin with a probe, wherein the probe reacts with accessible regions of cellular chromatin (page 4, lines 27-28); subsequently fragmenting the cellular chromatin to generate a collection of polynucleotide fragments (page 4, lines 30-34); and selectively cloning polynucleotide fragments that comprise a site of probe reaction (page 28, lines 6-7; page 50, lines 1-7). In certain embodiments, reaction of the probe with cellular chromatin results in polynucleotide cleavage at the site of reaction (page 5, line 29).

The cellular chromatin may be present in an isolated nucleus (page 44, lines 13-14) and, during certain steps of the method, the isolated nucleus may be embedded in agarose (page 52, lines 8-10). The probe used may be an enzyme, for example a nuclease (page 4, lines 27-28; page 5, line 29) such as DNAseI (page 26, line 32; page 49, lines 25-27) or a restriction enzyme (page 52, lines 5-6). Fragmentation can be achieved by the use of a restriction enzyme (page 49, lines 27-29) such as Sau3AI (page 50, lines 4-5; page 52, line 12).

VI. ISSUES ON APPEAL

1. Whether claims 123-152 are obvious over U.S. Patent No. 5,635,355 (hereinafter "Grosveld").

VII. GROUPING OF CLAIMS

Claims 123-152 are separately patentable, enabled and described by the application as filed. Therefore, these claims are divided into 30 separate groups:

(1) Claim 123: Independent claim 123 is drawn to a method for preparing a library of regulatory DNA sequences from a cell. The method comprises the steps of providing a cell nucleus, the nucleus including cellular chromatin, and contacting the nucleus with a first enzyme that reacts with accessible regions of cellular chromatin. Subsequently, the cellular chromatin is deproteinized and the deproteinized chromatin is contacted with a second enzyme to generate DNA fragments. The fragments generated are then contacted with a population of vector molecules, the vector molecules comprising a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends. Finally, polynucleotides comprising a DNA fragment ligated to a vector molecule are selected.

(2) Claim 124: Claim 124 is directed to the method of claim 123 and further specifies that the cell is selected from the group consisting of animal cells, plant cells and microbial cells.

(3) Claim 125: Claim 125 is directed to the method of claim 123 and further specifies that the first enzyme is a nuclease.

(4) Claim 126: Claim 126 is directed to the method of claim 125 and further specifies that the nuclease is DNase I.

(5) Claim 127: Claim 127 is directed to the method of claim 125 and further specifies that the nuclease is a restriction enzyme.

(6) Claim 128: Claim 128 is directed to the method of claim 123 and further specifies that the second enzyme is a restriction enzyme.

(7) Claim 129: Claim 129 is directed to the method of claim 128 and further specifies that the restriction enzyme is Sau3A I.

(8) Claim 130: Claim 130 is directed to the method of claim 129 and further specifies that the second end of the vector molecule is generated by digestion with BamH I.

(9) Claim 131: Claim 131 is directed to the method of claim 126 and further specifies that subsequent to the step of contacting the nucleus with the first enzyme, the DNase I ends are converted to blunt ends.

(10) Claim 132: Claim 132 is directed to the method of claim 131 and further specifies that the first end of the vector molecule is a blunt end

(11) Claim 133: Claim 133 is directed to the method of claim 132 and further specifies that the first end of the vector molecule is generated by digestion with EcoRV or SmaI.

(12) Claim 134: Claim 134 is directed to the method of claim 123 and further specifies that during the steps of contacting the nucleus with the first enzyme, deproteinization and contacting the fragments with the second enzyme, the nucleus is embedded in agarose.

(13) Claim 135: Claim 135 is directed to the method of claim 123 and further specifies that a plurality of different libraries of regulatory DNA sequences are prepared, wherein each library is obtained from a different cell.

(14) Claim 136: Claim 136 is directed to the method of claim 135 and further specifies that nuclei provided are obtained from cells at different stages of development.

(15) Claim 137: Claim 137 is directed to the method of claim 135 and further specifies that the nuclei provided are obtained from cells in different tissues.

(16) Claim 138: Claim 138 is directed to the method of claim 135 and further specifies that the nuclei provided are obtained from diseased cells and counterpart normal cells.

(17) Claim 139: Claim 139 is directed to the method of claim 135 and further specifies that the nuclei provided are obtained from infected cells and counterpart uninfected cells.

(18) Claim 140: Claim 140 is directed to the method of claim 135 and further specifies that the nuclei are obtained from cells that express a gene of interest at different levels.

(19) Claim 141: Claim 141 is directed to the method of claim 123 and further specifies that a plurality of different libraries of regulatory DNA sequences are prepared and, for each library, a different first enzyme is used.

(20) Claim 142: Claim 142 is directed to the method of claim 141 and further specifies that the different libraries are combined.

(21) Claim 143: Independent claim 143 is drawn to a method of for isolating a collection of polynucleotides comprising cellular regulatory sequences. The method comprises contacting cellular chromatin with a probe which reacts with accessible regions of cellular chromatin; subsequently fragmenting the cellular chromatin to generate a collection of polynucleotide fragments; and selectively cloning polynucleotide fragments containing a site of probe reaction.

(22) Claim 144: Claim 144 is directed to the method of claim 143 and further specifies that reaction of the probe with cellular chromatin results in polynucleotide cleavage at the site of reaction.

(23) Claim 145: Claim 145 is directed to the method of claim 143 and further specifies that the cellular chromatin in present in an isolated nucleus.

(24) Claim 146: Claim 146 is directed to the method of claim 145 and further specifies that during the steps of contacting and fragmenting, the isolated nucleus is embedded in agarose.

(25) Claim 147: Claim 147 is directed to the method of claim 143 and further specifies that the probe is an enzyme.

(26) Claim 148: Claim 148 is directed to the method of claim 147 and further specifies that the enzyme is a nuclease.

(27) Claim 149: Claim 149 is directed to the method of claim 148 and further specifies that the nuclease is a restriction enzyme

(28) Claim 150: Claim 150 is directed to the method of claim 148 and further specifies that the nuclease is DNase I.

(29) Claim 151: Claim 151 is directed to the method of claim 143 and further specifies that the step of fragmenting of cellular chromatin is by restriction enzyme digestion.

(30) Claim 152: Claim 152 is directed to the method of claim 151 and further specifies that the restriction enzyme is Sau3A1.

VIII. ARGUMENTS

1. The rejection

All pending claims were rejected in the Final Office Action (mailed April 13, 2004) under 35 U.S.C. § 103(a) (hereinafter "103") as allegedly obvious U.S. Patent No. 5,635,355 (hereinafter "Grosveld" or "the primary reference"), alone or in combination with various secondary references. In particular, claims 123-128, 130, 135, 143-145 and 147-151 were alleged to be obvious over Grosveld. Claims 129, 131-133 and 152 were rejected as allegedly obvious over Grosveld in view of the NEB catalog. Claims 136-142 were rejected as allegedly obvious over Grosveld in view of U.S. Patent No. 5,500,356 (hereinafter "Li"). Finally, claims 134 and 146 were rejected as allegedly obvious over Grosveld in view of U.S. Patent No. 6,444,421 (hereinafter "Chung").

In the Final Office Action, the Examiner pointed to one section of Grosveld (Column 8), which discloses the mapping of DNaseI hypersensitive sites (Column 8, lines 2-4), as allegedly teaching steps (a)-(d) of claim 123 and steps (a) and (b) of claim 143. The Examiner then selected two other sections of Grosveld (Column 15 and claim 1), disclosing the cloning of four fragments, each containing a DNase hypersensitive site, into a vector as allegedly teaching steps (e) and (f) of claim 123 and step (c) of claim 143.

The Examiner stated that, although Grosveld does not exemplify cloning of a DNaseI hypersensitive fragment into a vector, "Grosveld expressly teaches and suggests cloning of DNaseI hypersensitive site DNA fragments (see claim 1)"[2]. The rejection was also supported by the Examiner's contention that a library can comprise any "cloned set of nucleic acids[3]" apparently including multiple copies of the same cloned nucleic acid.

Appellants submit that the Examiner has failed to make out a prima facie case of obviousness because no combination of the references teaches or suggests each and every

[2] Office Action of April 13, 2004, page 4

[3] Office Action of April 13, 2004, pages 9-10.

element of the invention recited in claims 123-152. Further, there is simply no motivation within the references to arrive at the claimed invention.

1. *Prima facie* obviousness of claims 123-152 has not been established

The Examiner bears the burden of establishing a *prima facie* case of obviousness. *See, e.g., In re Ryckaert,* 28 USPQ2d 1955, 1956 (Fed. Cir. 1993); and *In re Oetiker,* 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). The reference must teach all the limitations of the claimed invention and, moreover, must suggest the desirability of arriving at the claimed subject matter. (*See, e.g., Amgen, Inc. v. Chugai Pharm. Co.,* 18 USPQ2d 1016, 1023 (Fed. Cir. 1991) stating that "hindsight is not a justifiable basis on which to find that the ultimate achievement of along sought and difficult scientific goal was obvious" and *In re Laskowski,* 10 USPQ2d 1397, 1399 (Fed. Cir. 1989) stating that "the mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.") Thus, the Board has previously acknowledged that disclosure of an isolated protein does not necessarily render obvious the same recombinantly produced protein. *See, e.g., Ex parte Goeddel,* 5 USPQ2d 1449 (BAPI, 1987).

Appellants argued, in Responses mailed on December 17, 2003 and February 26, 2004, that the cited passages from Grosveld disclose only the construction of a clone containing four DNase hypersensitive sites; but fail to disclose or suggest the construction of libraries, as claimed. Independent claims 123 and 143 both recite a series of steps which generate a large number of different DNA fragments (note the use of the plural "DNA fragments" and "polynucleotide fragments" in the claims) followed by a step for selectively cloning a subset of the fragments that are generated, such that a collection of clones containing different fragments is obtained. By contrast, Grosveld discloses, in one section, the mapping of DNase hypersensitive sites and, in another section, the construction of a single clone containing four DNase hypersensitive sites. Nowhere does Grosveld disclose or suggest the construction of a library comprising a collection of clones, each representing a different hypersensitive site.

Nonetheless, the Examiner rejected these arguments, maintaining - that a library can be any cloned set of nucleic acids and that, because Grosveld discloses a single clone containing multiple hypersensitive site-containing fragments, the claims are somehow obvious. In reality, the fact remains that Grosveld merely discloses a clone, not a library, and thus there is no *prima facie* case for the obviousness of the claimed methods for constructing libraries.s

(a) The term "library" refers to a collection of different nucleotide sequences

As mentioned above, Appellants responded to the rejection (as set forth in the Final Office Action) by pointing out that Grosveld discloses a clone, not a library, and pointed to their definition of library at page 46, lines 6-7 as referring to a pool of DNA fragments propagated in a cloning vector.[4] Appellants also pointed to a number of definitions in the art that were inconsistent with the Examiner's position that the definition of "library" includes multiple copies of the same sequence.[5]

With regard to the specification, Appellants again note that the specification clearly uses the term library in its conventional sense to refer to collections of different sequences. For instance, on page 46, lines 6-7, it is stated that "As used herein, the term "library" refers to a pool of DNA fragments that have been propagated in some type of a cloning vector." In addition, page 47, lines 5-6 states that "The libraries formed can represent accessible regions for a particular cell type or cellular condition," while page 45, lines 7-8 states that "Collections of accessible region sequences from a particular cell can be cloned to generate a library . . ."

Plainly, Appellants' specification uses terms such as "pool," "fragments," and "accessible regions" in the plural form to indicate that a library includes not only more than one nucleotide sequence, but, in addition, nucleotide sequences that are different from one another.

The definition of "library" in the specification is clearly consistent with the art-recognized use of the term as well. It is well known in the art of molecular biology that a nucleic

[4] Response After Final dated May 18, 2004, pages 3-4

[5] Response After Final dated May 18, 2004, pages 2-3

acid library is a collection of different nucleotide sequences. *See*, for example, Ausubel *et al.* (1987), copy attached to Response filed May 18, 2004 (Appendix B hereto):

> . . . a recombinant DNA library consists of a large number of recombinant DNA clones, *each one of which contains a different segment of foreign DNA*. (emphasis added)

See also Berger *et al.* (1987), copy attached to Response filed May 18, 2004 (Appendix C hereto):

> A library is a mixture of clones constructed by inserting either cDNA or fragments of genomic DNA into a suitable vector. The term *library* implies the existence of large numbers of different recombinants . . . (emphasis in original)

In sum, the specification and art as a whole clearly indicate that a library is a collection of different sequences, not simply multiple copies of the same sequence.

Finally, Appellants note that, in related application USSN 10/083,682 (a continuation-in-part of the present application), the Office has defined the term "library" as follows:

> . . . a library is interpreted as "an unordered collection of clones (i.e., cloned DNA from a particular organism)" [citation omitted]. Thus, the number of clones of polynucleotides in each library may vary.[6]

Thus, the Examiner's interpretation of the term "library" is incorrect (and inconsistent) and, to the extent that the Office asserts that a library is nothing more that a set of multiple copies of the same sequence is repugnant to the normal meaning of the term in the art.

Moreover, in a sincere effort to address the Examiner's concerns[7], Appellants amended the claims After Final to make explicit that the term library necessarily implies a collection of different sequences; however, the amendments were not entered. Appellants reiterate herein the

[6] USSN 10/083,682: Office Action dated September 9, 2004, page 6 (copy attached as Appendix D)

[7] Even though Appellants believed these concerns to be misplaced.

fact that this amendment did not raise new issues, but merely clarified an already-defined claim term.

To recap, the justification for disregarding Appellants' evidence that Grosveld fails to teach methods of preparing libraries as claimed is based on the Examiner's definition of a "library" as follows:

> A nucleic acid library is simply a cloned set of nucleic acids. So when Grosveld teaches cloning of the target nucleic acid into a vector, Grosveld is forming a library. (Final Office Action, page 9).

In fact, as pointed out in this Brief, and as previously pointed out in Appellants' responses, the term library is well known by those working in the field to refer to a collection of different sequences. The Examiner's definition of library is improper and, in fact, repugnant to the normal meaning of the term in the art. Indeed, it is this improper interpretation of the term "library" that has resulted in all of the rejections based thereon being erroneously maintained, as there is no teaching, suggestion or motivation within any of the cited references to support the rejection made by the Examiner. Additionally, the claims are patentable because of secondary considerations of non-obviousness; *e.g.*, a long-felt need.

(b) The primary reference does not teach or suggest any methods involving "libraries"

The pending claims are directed to methods of preparing libraries, using specific processes of cloning DNA fragments, as set forth in the first step(s) of the claims. For the reasons noted above, a library refers to a collection of different polynucleotide sequences.

There are absolutely no teachings by Grosveld regarding preparation of libraries in any way whatsoever. Rather, Grosveld states that hypersensitive sites may be "mapped" (col. 7, lines 59-63). The only cloning referred to in Grosveld involved construction of a single target sequence that, in certain cells, comprises a DNAse hypersensitive site. Cloning of "the target

sequence" (*i.e.*, a single sequence) into a vector results in the production of multiple copies of the same sequence, or what is normally referred to in the art as a clone. It does not produce a library of polynucleotide sequences in the same way that a building containing multiple copies of the same book for loan would not be considered a library.

Therefore, because Grosveld fails to disclose the construction of any type of library at all, let alone libraries of accessible sequences, as claimed, the primary reference, alone or in combination with any of the secondary references, cannot render any of the pending claims obvious. Accordingly, a *prima facie* case of obviousness cannot be established.

The steps and results of the claimed methods are precisely defined -- in the claims themselves, not in the references. None of the references teach methods of preparing libraries of accessible regions. Therefore, Appellants respectfully request that the rejection of these claims as allegedly obvious over the cited references be withdrawn, and that these claims be allowed.

Finally, it is noted that, since Grosveld's disclosure is directed to obtaining integration site-independent gene expression using DNase hypersensitive sites (as admitted by the Examiner[8]), it is aimed at a different problem than that of the presently-claimed subject matter, which is directed to genome-wide isolation and purification of regulatory sequences. Previously, regulatory sequences could not be isolated because they were destroyed in the process of being identified. Accordingly, there is no motivation for one of skill to look to Grosveld for guidance on how to simultaneously isolate and purify a plurality of regulatory sequences from a cell.

2. Additional Arguments Regarding Separately Grouped Claims

Each one of the preceding arguments is applicable to all of the separately grouped claims, *i.e.*, to each claim individually. For the sake of brevity, the arguments have been set out primarily as to independent claim 123. Claims 124-142 contain all the elements of claim 123 and are, therefore, patentable over the cited references for the reasons discussed in detail above. Similarly, claims 144 to 152 contain all the elements of claim 143 and are likewise patentable.

[8] Office Action of April 13, 2004, page 5, lines 13-17

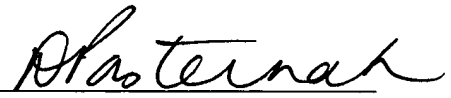
The dependent claims are also further limited in ways that are neither described nor suggested by the cited references, namely by further defining the elements of the claimed methods.

CONCLUSION

For the reasons stated above, Appellants respectfully submit that the pending claims are patentable over the art cited by the Examiner. Accordingly, Appellants request that the rejections of the claims on appeal be reversed, and that the application be remanded to the Examiner so that the appealed claims can proceed to allowance.

Respectfully submitted,

Date: September 30, 2004

By: 

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CLAIMS ON APPEAL

123. (previously presented): A method for preparing a library of regulatory DNA sequences from a cell, the method comprising:

(a) providing a cell nucleus, wherein the nucleus comprises cellular chromatin;

(b) contacting the nucleus with a first enzyme, wherein the first enzyme reacts with accessible regions of cellular chromatin;

(c) deproteinizing the cellular chromatin to generate deproteinized DNA;

(d) contacting the deproteinized DNA with a second enzyme to generate DNA fragments;

(e) contacting the DNA fragments obtained in step (d) with a population of vector molecules, wherein the vector molecules comprise a first end that is compatible with the first enzyme and a second end that is compatible with the second enzyme, under conditions favorable to ligation of compatible ends; and

(f) selecting polynucleotides comprising a DNA fragment ligated to a vector molecule.

124. (previously presented): The method of claim 123, wherein the cell is selected from the group consisting of animal cells, plant cells and microbial cells.

125. (previously presented): The method of claim 123, wherein the first enzyme is a nuclease.

126. (previously presented): The method of claim 125, wherein the nuclease is DNase I.

127. (previously presented): The method of claim 125, wherein the nuclease is a restriction enzyme.

128. (previously presented): The method of claim 123, wherein the second enzyme is a restriction enzyme.

129. (previously presented): The method of claim 128, wherein the restriction enzyme is Sau3A I.

130. (previously presented): The method of claim 129, wherein the second end of the vector molecule is generated by digestion with BamH I.

131. (previously presented): The method of claim 126, wherein, subsequent to step (b), the DNase I ends are converted to blunt ends.

132. (previously presented): The method of claim 131, wherein the first end of the vector molecule is a blunt end.

133. (previously presented): The method of claim 132, wherein the first end of the vector molecule is generated by digestion with EcoRV or SmaI.

134. (previously presented): The method of claim 123 wherein, during steps (b) – (d), the nucleus is embedded in agarose.

135. (previously presented): The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared, wherein each library is obtained from a different cell.

136. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from cells at different stages of development.

137. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from cells in different tissues.

138. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from diseased cells and counterpart normal cells.

139. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from infected cells and counterpart uninfected cells.

140. (previously presented): The method of claim 135 wherein, in step (a), nuclei are obtained from cells that express a gene of interest at different levels.

141. (previously presented): The method of claim 123, wherein a plurality of different libraries of regulatory DNA sequences are prepared and, for each library, a different first enzyme is used.

142. (previously presented): The method of claim 141, wherein the different libraries are combined.

143. (previously presented): A method for isolating a collection of polynucleotides comprising cellular regulatory sequences, wherein the method comprises:

(a) contacting cellular chromatin with a probe, wherein the probe reacts with accessible regions of cellular chromatin;

(b) subsequently fragmenting the cellular chromatin to generate a collection of polynucleotide fragments; and

(c) selectively cloning polynucleotide fragments of step (b) comprising a site of probe reaction.

144. (previously presented): The method of claim 143, wherein reaction of the probe with cellular chromatin results in polynucleotide cleavage at the site of reaction.

145. (previously presented): The method of claim 143, wherein the cellular chromatin in present in an isolated nucleus.

146. (previously presented): The method of claim 145 wherein, in steps (a) and (b), the isolated nucleus is embedded in agarose.

147. (previously presented): The method of claim 143, wherein the probe is an enzyme.

148. (previously presented): The method of claim 147, wherein the enzyme is a nuclease.

149. (previously presented): The method of claim 148, wherein the nuclease is a restriction enzyme.

150. (previously presented): The method of claim 148, wherein the nuclease is DNase I.

151. (previously presented): The method of claim 143 wherein, in step (b), cellular chromatin is fragmented by restriction enzyme digestion.

152. (previously presented): The method of claim 151, wherein the restriction enzyme is Sau3A1.

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 2

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Published by Greene Publishing Associates and Wiley-Interscience

John Wiley & Sons

New York • Chichester • Brisbane

09/844,501
Exhibit A
May 18, 2004

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Library of Congress Cataloging in Publication Data:

Current protocols in molecular biology. 2 vols.
1. Molecular biology—Technique. 2. Molecular biology—Laboratory manuals. I. Ausubel, Frederick M.

QH506.C87 1987 574.8'8'028 87-21033
ISBN 0-471-50338-X
ISBN 0-471-50337-1 (Vol. 2 binder)

Printed in the United States of America

10 9 8 7 6 5 4

Core + Supps. 1, 2, & 3

INTRODUCTION

The usual approach to isolating a recombinant DNA clone encoding a particular gene or mRNA sequence is to screen a recombinant DNA library. As described in Chapter 5, a recombinant DNA library consists of a large number of recombinant DNA clones, each one of which contains a different segment of foreign DNA. Since only a few of the thousands of clones in the library encode the desired nucleic acid sequence, the investigator must devise a procedure for identifying the desired clones. The optimal procedure for isolating the desired clone involves a positive selection for a particular nucleic acid sequence. If the desired gene confers a phenotype that can be selected in bacteria, then only the desired clone will grow under selective conditions, and it can be isolated in a rapid, effortless fashion. However, most eukaryotic genes and even many bacterial sequences do not encode a gene with a selectable function. Clones encoding nonselectable sequences are identified by screening libraries: the desired clone is identified either because it hybridizes to a nucleic acid probe or because it expresses a segment of protein that can be recognized by an antibody.

Screening libraries involves the development of a rapid assay to determine whether a particular clone contains the desired nucleic acid sequence. This assay is used first to identify the recombinant DNA clone in the library and then to purify the clone (see Fig. 6.0.1). Normally, this screening procedure is performed on bacterial colonies containing plasmids or cosmids or on bacteriophage plaques. To test a large number of clones at one time, the library is spread out on agarose plates (*UNIT 6.1*), then the clones are transferred to filter membranes (*UNIT 6.2*). The clones can be simultaneously hybridized to a particular probe (*UNITS 6.3* and *6.4*) or bound to an antibody (*UNIT 6.7*). When the desired clone is first identified, it is usually found among many undesirable clones; an important feature of library screening is the isolation of the desired clones (*UNITS 6.5* and *6.6*). Another method for identifying the desired clone involves hybrid selection (*UNIT 6.8*), a procedure in which the clone is used to select its mRNA. This mRNA is characterized by its translation into the desired protein.

To screen a DNA library, one must first devise the screening procedure. The next important choice is the selection of a recombinant DNA library. When choosing which library to screen the investigator should consider whether he or she wants to isolate clones encoding the gene or the mRNA sequence. cDNA clones will encode the mRNA

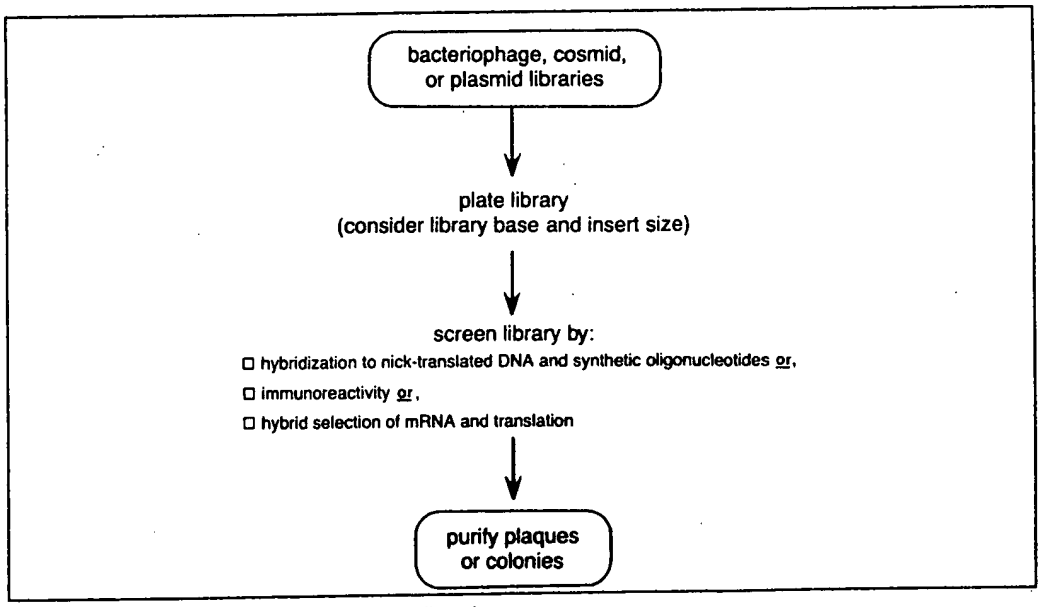

Figure 6.0.1 Flow chart for screening libraries.

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Methods in Enzymology

Volume 152

Guide to Molecular Cloning Techniques

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1987

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
Orlando San Diego New York Austin
Boston London Sydney Tokyo Toronto

09/844,501
Exhibit B
May 18, 2004

plaques *in situ* to replace those that have been removed during the preparation of filters or to increase subsequent hybridization signals.

1. Prepare an overnight culture of plating bacteria (this volume [13 or 17]). You will need 400 ml of culture for 20 filters.
2. Collect bacteria by centrifugation and resuspend them in an equal volume of fresh LB + 10 m*M* $MgSO_4$. General methods for handling λ are found in this volume [13].
3. Label the filters and mark them asymmetrically, with a black ballpoint pen, on the side that will be in contact with the plaques.
4. Dip the filters in the bacterial cell suspension and allow them to air dry briefly.
5. Lay the filters on the surface of the plates containing plaques. Transfer the orientation marks to the agar plate. The techniques in step 4 of the alternate procedure can be employed.
6. Prepare additional filter copies, if desired. Be sure to transfer orientation marks from agar to filter. A light box is a useful aid here.
7. Lay copy filters, phage plaque side up, on fresh LB + Mg^{2+} plates and incubate, inverted, at 37° overnight.

During the overnight growth at 37° the plaques infect the growing *E. coli*, leading to a substantial amplification of phage DNA. After this amplification, it is usually not necessary to hybridize two sets of filters to avoid false positives.

8. Remove the filters from the plates, air dry for at least an hour and process filters as in step 16, omitting the 10% SDS treatment as described above. Store plates, inverted, sealed in Parafilm at 4°. The filters are ready for prehybridization (this volume [45]).

[45] Screening Colonies or Plaques with Radioactive Nucleic Acid Probes

By GEOFFREY M. WAHL and SHELBY L. BERGER

Colony or plaque hybridization is a technique for screening replicated material *in situ* on filters with labeled probes.[1-5] The probes most com-

[1] M. Grunstein and D. S. Hogness, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3961 (1975).
[2] M. Grunstein and J. Wallis, this series, Vol. 68, p. 379.
[3] W. D. Benton and R. W. Davis, *Science* **196**, 180 (1978).
[4] D. Hanahan and M. Meselson, *Gene* **10**, 63 (1980).
[5] D. Hanahan and M. Meselson, this series, Vol. 100, p. 333.

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monly used are nucleic acids or antibodies. Here we will describe techniques for using nucleic acids to analyze libraries generated in either phages or plasmids. The use of antibodies for screening libraries can also be found in this volume [50, 51].

A library is a mixture of clones constructed by inserting either cDNA or fragments of genomic DNA into a suitable vector. The term *library* implies the existence of large numbers of different recombinants, only one or a few of which are of immediate interest to the investigator. The desired clone is located by performing the following steps: (1) transfected bacteria or phage are grown on master plates (or filters) and replica plated; (2) the original plates called *master plates* are preserved while the replicas, hereafter called *filters*, are processed; (3) phage are disrupted or bacteria are lysed *in situ* on filters; (4) DNA is bound to the filter while RNA is hydrolyzed; (5) the resulting partially denatured DNA is hybridized to sequences able to bind specifically to the desired insertions. (6) Because the configuration of DNA on the filter replicas matches the configuration of live bacteria or phage on the master plates, DNA on replicas which binds to the probe (so-called positive signals) can direct the investigator to the bacterial colony or phage plaque from which the DNA was derived; (7) the positive colony or plaque is then purified and grown in quantity for further analysis.

Chapters [44] and [18] describe steps 1–4 for plasmid or λ libraries and cosmid libraries, respectively. Here we will focus on steps 5–7.

Colony hybridization is a rapid but inexact procedure aimed at calling attention to clones worthy of serious consideration. Falsely positive clones are therefore not uncommon. To some extent these can be reduced by the following: (1) use both negative control filters and, if possible, positive control filters; (2) screen duplicate filters of each master plate; and (3) prepare probes carefully.

To satisfy the requirements of point 1, it is advisable to include clones containing the vector without an insert or containing an irrelevant insert. The latter is particularly important when fragments bearing homopolymer "tails," usually composed of dG on one strand and dC on the other, are screened; the GC-rich regions on either end of the insert can hybridize to GC-rich probes and cause spurious positive signals. Thus, the use of known negative recombinants acts as a means for detecting unwanted cross-hybridization of the probe to vector and host DNA (which are also present) and also serves to establish the intensity of a background signal, one that should be ignored. Since intensities are relative, a genuine positive signal is needed for comparison. If there are no known positive clones, one can always clone the probe itself and create a positive recombinant. Such engineered positive colonies or plaques are rarely perfect

The claims, as currently written, are drawn to library or libraries of polynucleotides comprising polynucleotides corresponding to the accessible regions of cellular chromatin obtained by the method of claim 3. Each of these claims is directed to a genus comprising any library of polynucleotides comprising polynucleotides corresponding to the accessible regions of cellular chromatin obtained by the method of claim 3. Note that absent an explicit definition in the specification of the term, a library is interpreted as "an unordered collection of clones (i.e., cloned DNA from a particular organism)" (see Biotech Life Science Dictionary, URL: http://biotech.icmb.utexas.edu/search/dict-search.phtml?title=library). Thus the number of clones of polynucleotides in each library may vary. Further, since the probes used in claim 3 may be a chemical, an enzyme or an antibody, each of which may react with, and thus mark, different polynucleotides, the claimed genus comprises different species of libraries comprising different polynucleotides.

A description of a genus may be achieved by means of a recitation of a representative number of species, falling within the scope of the genus, or by means of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. Regents of the University of California v. Eli Lilly & Co., 119 F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). In the instant case, however, the specification does not describe the structure (i.e. the sequences of each clone of a library) of any species, nor does it describe any structural feature (i.e. the sequence of each clone in a library) common to the members of the genus. No common structural attributes identify the members of the genus. While the specification gives example of how to make a library (see pages 113-116), it does not describe the structure of the library or libraries made. The general knowledge and level of skill in

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